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Collembola Predation on Nematodes[1]

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INTRODUCTION

Collembola are wingless hexapods that make up a large portion of the fauna of any soil habitat that contains decaying vegetation and adequate moisture. Although it has been known for some time that populations of Collembola may reach very high densities, the interactions between this group and the entire community are not well understood.

One of the most significant interactions of any group of organisms with its environment is in the flow of energy or in the food web of the community. This includes the predators of that group of organisms as well as what they, in turn, eat. This study was designed to focus on one aspect of that involvement, the food of Collembola, and more specifically on the use of nematodes as a food by Collembola.

Collembola are usually considered herbivores (Englemann, 1966, 1968) or fungus and detritus feeders (Christiansen, 1964). Christiansen also lists bacteria, pollen, fungus spores, and occasionally live animals as minor food sources. The possibility that Collembola are predators of soil nematodes has been cited on a few previous occasions. Brown (1954) reported observing an isotomid eating nematodes from the inner surface of the lid of a chamber that contained an ant culture. The Collembola consumed many live, active nematodes in a short time.

Murphy and Doncaster (1957) reported seeing *Onychiurus armatus* Tullberg feeding on the mature female cysts of *Heterodera cruciferae* Franklin, and presented circumstantial evidence that this might also be true for a field situation. Doncaster (1926) reported *Achorutes, Folsomia,* and *Isotoma* feeding on *Heterodera* cysts. The contents of 1 or 2 immobile cysts were consumed over a period of a day or more; Murphy and Doncaster (1957) reported that they found no evidence that active nematodes would be eaten.

With these reports in mind the first purpose of this study was to see whether the results of the experiments could be duplicated. A method of duplication was brought to my attention by Dr. W. L. Brown, Jr., of Cornell University. In his laboratory were a number of ant cultures that were fed fly pupae. Many small nematodes were observed on the pupae in one culture; another culture was contaminated with Collembola. When Collembola from the second culture were added to the first, they soon began to feed on the nematodes until most of them were eaten. Collembola from the contaminated ant culture were used to start a new culture. Specimens from this culture were later identified by Dr. Kenneth Christiansen, Grinell, Iowa, as *Entomobryoides dissimilis* (Moniez). Unfortunately, the nematode culture started from the infected ant culture became contaminated with mites and was discarded before identification was possible.

These preliminary observations provided confirmation of the report by Brown (1954), although for a different group of Collembola. The second and major purpose of this work then became the investigation of the extent of Collembola activity and the possible nutritional significance of this behavior.

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[1]Adapted from a thesis submitted to the faculty of the graduate school of Cornell University, August 1968, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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GENERAL METHODS AND MATERIALS

Rearing and Handling of Collembola

Several techniques have been described for culturing Collembola (Edwards, 1955; Goto, 1960; Sharma and Kevan, 1963; and von Torne, 1964). Central to all of these is the use of a substrate of plaster of Paris mixed with powdered charcoal. Culture chambers were selected to rear large numbers of Collembola, to reduce contamination by mites, and to provide the ease necessary for handling large numbers. These chambers, constructed from wide-mouth pint canning jars (12 cm deep, 7.5 cm top diameter, and 6 cm bottom diameter), were filled ⅓ with charcoal-plaster. A mixture of plaster of Paris and powdered charcoal, 9 to 1 by volume, was prepared. This dry mixture was combined with water, 3 to 2 by volume, and poured into the containers to set. The lids of the jars were fitted with a small piece of plastic tubing, which was then plugged with cotton.

Bakers' dried yeast was used as food for stock cultures (Goto, 1960; Sharma and Kevan, 1963). The yeast was placed on small pieces of charcoal-plaster, which could be removed easily if the yeast became contaminated. Water was provided irregularly when the food was changed. All cultures were kept in an incubator that had a daily period of 10 hours of low intensity light. The temperature and humidity fluctuated with a regular daily cycle.

Entomobryoides dissimilis and *Sinella caeca* (Schott) were maintained in stock cultures. *S. caeca* reproduced more rapidly and consistently, and was easier to handle than *E. dissimilis*. Therefore, *S. caeca* was used for studies of life cycle and growth, and was maintained in large numbers. Other species of Collembola were collected from their natural habitats and not maintained in cultures.

Because life history and growth rate studies required large numbers of Collembola of known ages, it was necessary to devise methods of handling them quickly and efficiently. An aspirator, constructed from a section of rubber tubing with 3-inch pieces of glass tubing in both ends, and with the inserted ends covered with a piece of silk cloth, was used for transferring adults. This was not practical for transferring immatures, especially first-instar *S. caeca*. Many specimens were injured by the force of being moved even a few inches in an aspirator, or they were caught in the cloth. Therefore, a miniature aspirator was devised.

This was constructed from 2 pieces of glass tubing, 6–7 cm long, 1 piece with an inside diameter of 4 mm and the other with an outside diameter of 3 mm. The larger diameter tubing was drawn out to resemble a pipette with a hole of about 1 mm at its tip. A ring of epoxy resin was put around one end of the smaller tubing, and a piece of fine nylon with holes 35μ square was placed over the same end. This was carefully inserted into the larger tubing as far as it would go. More epoxy was added where the smaller tubing protruded from the larger, and gentle suction was applied to the pipette end to draw the epoxy into the larger tubing, making a closed seal. The epoxy was then permitted to harden. When this ensemble was inserted into the end of a length of rubber tubing, the resulting aspirator carried the Collembola immatures 15–20 mm, while providing a soft, fine surface on which the specimens were neither damaged nor caught.

Collembola were transferred by the appropriate aspirator to a 1-dram shell vial for counting. Up to 15 Collembola could be counted directly, but larger groups were first anesthetized with CO_2. Edwards and Patton (1965) showed that high levels of CO_2 may have a detrimental effect on insects. To avoid this effect, CO_2 from a cylinder was mixed with air by passing it through a Bunsen burner (Patton, Edwards, and Gilmore, 1968). The air intake was kept at a constant opening and the CO_2 was turned on using the needle-valve adjustment, which was always opened one full turn. The air–CO_2 mixture was delivered by a length of flexible plastic tubing to the shell vial containing Collembola. This was fitted with a short piece of hard plastic tubing that was inserted into the shell vial. This delivery tube was adjusted so that it protruded only ½ inch into the vial, thus preventing injury to the Collembola on the bottom. The low concentration of CO_2 that was delivered by this apparatus was sufficient to quiet the Collembola for counting.

Collembola eggs were handled to permit 1000 or more to be transferred per hour. To obtain eggs that were as clean as possible, new culture jars were prepared each time that eggs were needed. Because Collembola adults have a tendency to drop their eggs into holes or under debris, the surface of the plaster was made smooth by adding a small amount of fluid charcoal-plaster to the freshly prepared jars after the first layer was firm. When the second layer was completely set, 250 to 500 adult *S. caeca* and 3 small charcoal-plaster chips containing yeast were added. The culture jars were then stored in the incubator.

Two days later, and every second day up to 10 days, all eggs were removed from the surface with a fine camel's hair brush. These were washed from the brush by dipping it into water in a spot plate. After the eggs had been transferred, the water was changed several times using a pipette that was drawn out to a diameter slightly larger than the eggs. This removed debris and pieces of feces that were inevitably transferred with the eggs. The eggs were then

transferred by pipette to a hatching jar (capacity 200 ml) that contained a layer of partially dried charcoal-plaster. The charcoal-plaster absorbed the excess water leaving the clean eggs on the surface. Using this technique, up to 95% of the eggs remained viable and eventually hatched. The young emerged between the 8th and the 10th day after the eggs were transferred. Thus, on the 10th day young could be transferred to test chambers by the miniature aspirator, providing first-instar young for which the age was known to within 48 hours.

Rearing and Handling of Nematodes

Bacteria-feeding nematodes were cultured on decaying mashed potatoes. The medium was prepared by stirring 200 ml of commercial dry potatoes (such as that manufactured by Borden's) into 400 ml of rapidly boiling water. Two teaspoons of this mixture were placed in the bottom of a 200 ml jar, the lid of which had been fitted with a small piece of plastic tubing that was plugged with cotton. After the jars were autoclaved at 15 pounds pressure for 15 minutes and then cooled to 20°C, inoculum was added. Three species of bacterial feeders were cultured in this manner. The first, an unidentified diplogasterid designated EA, was obtained from soil and dead ants taken from an ant culture at Cornell University. This species, along with *Diplogaster* sp. and *Panagrellus* sp. were maintained by periodically transferring a small portion of an old culture to freshly prepared potatoes.

Two species of fungus-feeding nematodes, *Neotylenchus linfordi* Hechler 1962, and *Aphelenchus* sp., were maintained in stock culture. These were kept in the culture jars described on a medium of 1/5 potato dextrose agar and 4/5 water agar (2% agar in water). New cultures were established by transferring a small piece of medium from an old culture. Original cultures of these and the 2 species named previously were obtained from the Nematology Laboratory of Cornell University.

Nematodes were extracted from the culture medium by the pie-pan modification of the Baermann funnel, as it is used in the Cornell Nematology Laboratory. Such extractions provided large numbers of active nematodes, with a minimum of extra debris. At no time in my studies were sterile or absolutely clean nematodes used. The volume of any debris was, however, quite small compared to the total volume of nematodes.

EVIDENCE THAT COLLEMBOLA EAT NEMATODES

Methods

When enough of one species of Collembola were available, nematodes were extracted from stock cultures and suspended in tap water. A surplus of suspension was used so that there was more than twice the total volume needed. The suspension was maintained by a magnetic stirrer, using a constant speed throughout the preparation for any one experiment. Five ml portions were withdrawn from a depth of ½ to ¾ of the total depth, as close as possible to the same spot. Each portion was then filtered through a disk of charcoal-plaster using a vacuum pump and a filtering flask. The disk was held in a rubber stopper which had a hole the diameter of the disk. The disks, 6 mm thick and 27 mm in diameter, had a shallow depression 1 cm in diameter in the center. The nematode suspension was slowly dropped into the depression as the water was filtered off. This process left the nematodes exposed on the surface of the disk and provided a series of disks with statistically similar numbers of nematodes.

The disks were then placed in separate petri dishes (60 mm, plastic). Dishes were randomly selected as controls or experimentals and the appropriate Collembola were added to the experimental dishes. Each dish was placed in a plastic box which contained a layer of the charcoal-plaster on the bottom, and the box lids were sealed with tape. This arrangement maintained an adequate humidity and made possible a simple means of determining whether the presence of Collembola made any difference in the number of nematodes remaining on the disks.

The boxes were stored in the incubator for a period of up to 72 hours, after which the Collembola were removed. Any nematodes that remained on the disks were washed into the bottom of the petri dish. The nematodes were then counted using an ocular grid. Counts were made at 5 different areas on the disk, 1 near the center and the others two thirds of the way to the edge in 4 directions. The average of the 5 counts was then used to obtain an estimate of the total number of nematodes in the dish. Estimates from experimental dishes were compared with those from control dishes and differences were assumed to indicate that the Collembola had eaten the nematodes. Differences between estimates from experimental and control dishes were also used to calculate the number of nematodes eaten by a single Collembola in 24 hours.

Because this method puts the nematodes in an unnaturally exposed position, a second method was used which

provided an environment with more natural protection. A standard volume of nematode suspension was added to a 60 mm petri dish that contained vermiculite or a mixture of fine soil and vermiculite. Beneath the soil and vermiculite was a layer of charcoal-plaster 4 mm thick. Excess water was absorbed by the charcoal-plaster, producing a humid environment in which both Collembola and nematodes could move around the soil particles and between pieces of mica. After several hours to permit the nematodes to move throughout the substrate, dishes were randomly distributed among experimentals and controls, Collembola were added, and the dishes were stored in the incubator. Following a period of exposure the nematodes were extracted from the soil and vermiculite by the pie-pan technique. Any nematodes extracted were counted and comparisons between controls and experimental dishes were made.

Species of Collembola That Ate Nematodes

Twelve species of Collembola representing 4 families were obtained in sufficient numbers for these tests. They were identified as *Hypogastrura packardi* (Folsom), *Sminthurides* sp., *Sinella caeca, Entomobryoides dissimilis, Willowsia nigromaculata* (Lubbock), *Tomocerus vulgaris* (Tullberg), *Tomocerus flavescens* (Tullberg), *Proisotoma schaefferi* (Krausbauer), *Folsomia candida* (Willem), *Isotomurus palustris* (Mueller), *Isotoma viridis* (Bourlet), and *Isotoma cinerea* (Nicolet). Identifications were made by myself, except for *E. dissimilis*. Specimens of all species were mounted in Salmon's polyvinyl alcohol, type MA2 (Salmon, 1951). A number of specimens of each species were deposited with the Cornell University insect collection, under the lot number 991, with sublots assigned in the order listed as 3, 6, 2, 1, 8, 11, 12, 7, 9, 10, 5, and 4.

Because *E. dissimilis* and *S. caeca* were observed feeding on nematodes they were used as positive controls along with several other species. They were also used for other portions of this work. Therefore, separate data are not presented for them. For the remaining 10 species, all but 3 of the differences between control and experimental treatments are highly significant (table 1), and only 1 is not significant at the 0.05% level of confidence. This indicates that the remaining 8 species did remove nematodes from the charcoal-plaster disks, and that it is probable that *P. schaefferi* and *W. nigromaculata* also removed nematodes from the disks.

It is possible that the reliability of the tests using these latter 2 species was adversely affected by the conditions of the test chamber. Many of them and the *Sminthurides* sp. died during the test. Since Collembola can live without food for some time, considerably longer than the 72 hours maximum for these tests, it is likely that some condition in the chambers other than food was not favorable.

Species of Nematodes Eaten

The preceding material indicates that the nematodes EA, *Diplogaster* sp., and *Panagrellus* sp. are eaten under the conditions provided in the laboratory (table 1). Four other nematodes were available in sufficient numbers for use in the charcoal-plaster disk method. These were *Neo-*

Table 1. Nematodes remaining on charcoal-plaster disks after exposure to predation by Collembola

Collembola	Nematodes per unit area = 16 mm²†			Standard deviation (pooled)	LSD‡ α.05 α = .01
	No Collembola control (n)	Positive control (n)	Experimental (n)		
H. packardi§	34.7 (2)	—	6.7** (3)	4.66	8.6 14.4
I. cinerea§	34.7 (2)	—	9.1** (3)	4.66	8.6 14.4
I. viridis‖	17.5 (6)	0.2 (5) ¶	3.8** (4)	1.52	1.7 2.5
Sminthurides‖	17.5 (6)	0.2 (5) ¶	13.9** (5)	1.52	1.6 2.4
P. schaefferi‖					
10 per dish	38.2 (5)	3.4 (5) ¶	36.8 (5)	4.80	5.3 7.8
20 per dish	38.2 (5)	3.4 (5) ¶	30.5* (5)	4.80	5.3 7.8
W. nigromaculata‖	86.0 (5)	31.9 (4) ††	67.5* (6)	15.94	17.2 25.9
F. candida‡‡					
10 per dish	184.2 (5)	—	135.2** (5)	12.30	13.9 20.9
20 per dish	184.2 (5)	—	89.7** (5)	12.30	13.9 20.9
I. palustris‡‡	72.2 (5)	2.8 (5) ††	26.0** (5)	11.25	12.7 19.1
T. vulgaris‡‡	63.4 (5)	—	6.6** (5)	9.40	11.1 17.2
T. flavescens‡‡					
20 per dish	113.8 (5)	—	64.3** (5)	11.15	13.1 20.0

†Total area of the dish equals 1963.5 mm².
‡Difference between the experimental and the no Collembola control greater than the LSD value indicates significance. (Steele and Torrie, 1960).
§EA nematode used as prey.
‖*Diplogaster* sp. used as prey.
¶*E. dissimilis* used as positive control.
††*S. caeca* used as positive control.
‡‡*Panagrellus* sp. used as prey.

Table 2. Nematodes remaining on charcoal-plaster disks after exposure to predation by Collembola

Nematode	Nematodes per unit area = 67.24 mm²†			Standard deviation (pooled)
	Control	*E. dissimilis*	*S. caeca*	
N. linfordi	97.8	1.0	0.8	5.4
Aphelenchus sp. . .	161.7	2.4	0.2	14.8
Tylenchus sp. . . .	37.2	1.3	0.1	4.6
D. dipsaci	126.4	0.8	0.1	2.6

†Total area of the counting dish equals 1963.5 mm²; multiply numbers given by 29.2 to obtain an estimate of the total number of nematodes per dish. Five replicates were averaged for each treatment. Since the differences were so large no statistical calculations were made.

tylenchus linfordi; Aphelenchus sp.; *Ditylenchus dipsaci* (Kuhn), the stem nematode which is endoparasitic on onion bulbs, among other plants; and *Tylenchus* sp., which is found around the roots of many plants (Mai, Lyon, and Kruk, 1968). Considerable numbers of each of these 4 species were eaten by both *E. dissimilis* and *S. caeca* (table 2).

Three species of nematodes that were not available in sufficient numbers for the preceding tests were presented to Collembola and observed to see if feeding took place. These were *Heterodera trifolii* Goffart 1932. *Criconemoides* sp., and *Aphelenchoides ritzemabosi* (Schwartz). Both *E. dissimilis* and *S. caeca* were observed biting the edges of broken cysts of *H. trifolii,* but since it could not be determined whether any of the cuticle or contents of the cysts were removed, evidence that they ate this species is lacking. *E. dissimilis* was observed voraciously ingesting *Criconemoides.* Only a few of this species were ingested by *S. caeca,* although several were picked up, manipulated in the mouthparts, and then returned to the charcoal-plaster surface. *A. ritzemabosi* was presented to *S. caeca* only. One hour after Collembola were added to the culture tube containing the nematodes many adults and several immatures were observed feeding on the nematodes. One adult consumed 12 nematodes in 10 minutes.

Of the 10 species of nematodes tested, 8 were eaten by *S. caeca* and 9 were eaten by *E. dissimilis.* The nematodes not readily eaten were those with a thick cuticle. Nonetheless, the evidence does not eliminate the possibility that contents of the cysts of *H. trifolii* were removed.

Numbers of Nematodes Eaten

Four series of tests were conducted involving *S. caeca* and *E. dissimilis* feeding on *Panagrellus* sp. to investigate the number of nematodes that a Collembola could eat. Enough disks were prepared so that controls and several groups of experimentals were possible, each group with a different number of Collembola. Differences between the number of nematodes in control dishes and in experimental dishes after 24 hours exposure to predation were used to calculate the average number eaten per Collembola. In all cases large numbers of nematodes were eaten in a 24-hour period, from 78 to 386 for *S. caeca,* and from 589 to 2427 for *E. dissimilis* (table 3).

While these data indicate that Collembola may consume large numbers of nematodes, it is not possible to

Table 3. Number of Panagrellus eaten by *S. caeca* and *E. dissimilis* in 24 hours

Collembola (n) ‡	Nematodes remaining in dish†		Nematodes eaten per Collembola
	Control (±Sd)	Experimental (±Sd) §	
E. dissimilis (5)	24044 (±2280)	11911 (±1381)	2427
E. dissimilis (10)	24044 (±2280)	6581 (±2707)	1746
E. dissimilis (20)	24044 (±2280)	490 (±196)	1178
E. dissimilis (40)	24044 (±2280)	22 (±17)	600
E. dissimilis (5)	10012 (±1074)	4393 (±2584)	1124
E. dissimilis (10)	10012 (±1074)	1890 (±280)	812
S. caeca (20)	8402 (±925)	6787 (±798)	81
S. caeca (40)	8402 (±925)	4069 (±603)	108
S. caeca (60)	8402 (±925)	2061 (±990)	106
S. caeca (80)	8402 (±925)	2160 (±1872)	78
S. caeca (20)	26695 (±2586)	18970 (±1112)	386
S. caeca (40)	26695 (±2586)	14705 (±1385)	300
S. caeca (60)	26695 (±2586)	10361 (±2988)	272
S. caeca (80)	26695 (±2586)	9139 (±2449)	219
E. dissimilis (10)	26695 (±2586)	17061 (±2293)	963
E. dissimilis (20)	26695 (±2586)	13360 (±2118)	667
E. dissimilis (40)	26695 (±2586)	3112 (±2313)	589

†Results from 5 replicates were averaged for each treatment. Estimates for each replicate were obtained from the average of 5 counts per dish.
‡N = the number of Collembola per experimental dish.
§All experimental averages differ significantly from the corresponding controls.

calculate an average number eaten per Collembola. Conditions may have been somewhat different for each series of tests. Within a single test, such factors as crowding and availability of nematodes may have affected the apparent number consumed, as indicated by the decrease in the number eaten as the number of Collembola per dish increased (table 3). It is also probable that more nematodes would have been eaten in the dishes containing the higher number of Collembola had more been available.

Nematodes Eaten in a Soil Environment

All experimental averages for these tests were significantly different from the corresponding controls (table 4). This indicates that the Collembola removed nematodes from a situation that provided some natural protection to the nematodes. The number eaten was, however, much less than the number eaten from the charcoal-plaster disks.

Table 4. Nematodes extracted from soil-vermiculite or vermiculite after exposure to predation by Collembola

Collembola (no. per dish)	Exposure (hrs.)	Nematodes per 16 mm²†		Standard deviation (pooled)	Nematodes eaten per Collembola in 24 hours
		Control (n)	Experimental (n)		
E. dissimilis (20)	68	28.0 (4)	15.9*‡ (5)	9.0	26.3
S. caeca (50)	68	28.0 (4)	9.6** (4)	9.0	16.0
S. caeca (40)	24	34.5 (5)	21.8* (5)	9.9	39.0
S. caeca (40)	72	119.3 (6)	79.0** (6)	16.4	41.2
S. caeca (40) §	72	35.6 (5)	12.3** (5)	11.1	23.8

†Total area of counting dish equals 1963.5 mm²; multiply numbers given by 122.7 to obtain an estimate of the total number of nematodes.

‡Significance determined by LSD test (Steele and Torrie, 1960).

§Vermiculite only.

FEEDING BEHAVIOR OF COLLEMBOLA

Many nematodes of various sizes were found to have moved onto the glass sides of the culture tube in the culture containing *A. ritzemabosi*, to which *S. caeca* was added. Since the Collembola could walk upside down on the glass, it was possible to observe their behavior as they fed on the nematodes.

The Collembola did not move onto the glass and, hence, did not discover the nematodes until 1 to 2 hours after they were introduced. After this time both adult- and intermediate-size specimens were observed feeding on the nematodes. Smaller specimens were observed searching in a manner similar to the adults, but none were seen ingesting a nematode. As the Collembola hunted rapidly over the tube, they moved their mouthparts close to the surface and held their antennae high and parallel, arched in front of their heads. A nematode was not eaten until the mouthparts actually contacted it. Even if an individual touched a nematode with one of its tarsi, no change in searching behavior was observed unless the mouthparts also touched it. However, all nematodes touched by the mouthparts during the period of observation were eaten. When a nematode was touched, the Collembola quickly bit into it and drew in a portion. As much as half of the largest nematodes could be drawn in from one end, resembling the manner in which some persons eat spaghetti. All parts of the nematode were removed from the surface before the Collembola moved on, unless it was disturbed by another Collembola. Even if disturbed, the first specimen usually returned to the spot and again began to feed on the same nematode. Such disturbances often appeared to be contests, in which the two Collembola struck each other with their antennae briefly and chased each other until one retreated.

Evidence was also obtained that the Collembola preferred the nematodes over bakers' yeast. Bakers' yeast has been used as food for stock cultures in my work, as well as that of many other researchers, and many successive generations have been reared on this food alone. It thus appears to provide a nutritionally adequate diet. To determine if nematodes would be eaten in the presence of yeast, several dishes containing a disk with yeast, in addition to the disk with nematodes, were included in the previous tests dealing with the number eaten. The average number of nematodes remaining in such dishes were compared with the averages from dishes with the same number of Collembola, but with nematodes as the only food.

Seven such comparisons are possible (table 5). Only 1 comparison resulted in a difference that is significant at the 0.05% level of confidence. In 5 of the other 6 comparisons

Table 5. Nematodes remaining after exposure to predation by Collembola in the presence and absence of yeast as an alternate food

Collembola (no. per dish)	Nematodes remaining in dish per unit area			LSD† $\alpha = .05$
	Control (±Sd)	With yeast (±Sd)	No yeast (±Sd)	
40 *S. caeca*‡	464 (±44.7)	135 (±24.3)	81 (±17.5)	65.9
10 *E. dissimilis*§	196 (±24.4)	49 (± 9.1)	54 (±22.1)	15.8
5 *E. dissimilis*§	82 (± 8.7)	32 (±14.3)	36 (±21.1)	14.3
10 *E. dissimilis*§	82 (± 8.7)	13 (±11.1)	15 (± 2.2)	14.3
20 *S. caeca*§	68 (± 7.5)	53 (±16.1)	55 (± 6.5)	10.5
40 *S. caeca*§	68 (± 7.5)	45 (± 3.9)	33* (±4.9)	10.5
60 *S. caeca*§	68 (± 7.5)	16 (± 6.9)	17 (± 8.0)	10.5

†Difference between no yeast and with yeast average greater than the LSD value indicates that the Collembola removed fewer nematodes if yeast was present as an alternate food.

‡Unit area equals 67.2 mm²; multiply by 29.2 to obtain the total. Three replicates each treatment.

§Unit area equals 16 mm²; multiply by 122.7 to obtain the total. Five replicates each treatment.

the number of nematodes eaten was actually greater in the presence of yeast, although these differences are not statistically significant. It thus appears that there is little difference in the number of nematodes eaten, whether yeast is present or not. When dishes containing both yeast and nematodes were opened after the period of exposure, the Collembola were frequently congregated around the disk that had held the nematodes. The yeast appeared to be undisturbed, although I could not determine whether small amounts had been removed. Thus the nematodes were rapidly eaten before any noticeable amount of the yeast had been consumed.

NUTRITIONAL SIGNIFICANCE OF NEMATODES IN DIET OF COLLEMBOLA

Methods

Three approaches were used in an investigation of the possible significance of nematodes in the diet of Collembola. These were, first, to determine whether nematodes are digested by Collembola, and if so, how rapidly. Second, to investigate the effect of a strictly nematode diet on the fecundity of Collembola. Third, to determine the effect of a nematode diet on the growth of Collembola through 1 generation.

Nematodes were presented to Collembola in 2 ways to determine whether they were digested. In the first method a number of specimens that had had no food for several days were placed in the bottom of a petri dish. This was covered with a lid from a petri dish in which EA nematodes had been cultured on fly pupae. When a culture of this type was more than 2 weeks old, many immature nematodes had migrated to the lid. These dishes were watched; when a Collembola was observed feeding, the number of nematodes ingested were counted and the time from the first observation of feeding was recorded. In several instances it was not possible to time the feeding, nor to count the number eaten. Such Collembola were, however, observed to be eating nematodes or were from a group of specimens, several of which had been observed ingesting nematodes. At the end of a short period the specimen was removed, killed, and placed in a drop of glycerine on a slide. The gut contents were separated from the abdomen, and the whole mount was squashed under a coverslip and studied for evidence of the presence of nematodes.

In the second method, nematodes were presented to Collembola on disks of charcoal-plaster. Six disks were put in separate dishes with 10 *E. dissimilis* each; another 6 were kept as controls. After 13 hours, 3 of the controls and 3 of the experimental dishes were processed. After 24 hours the remaining 6 dishes were processed. When the dishes were opened the Collembola were removed and killed in hot water. Five were placed in a drop of glycerine on a slide, dissected, and studied as before to see if any evidence of nematodes remained. Any nematodes remaining on the disks were washed into the petri dish and counted in order to determine how many had been removed.

Culture jars constructed from 200 ml baby-food jars were used to test the effect of a nematode diet on the fecundity and growth rate of Collembola. Short pieces of plastic tubing were glued into holes in the lids of these

jars and the tubing was plugged with cotton. A layer of charcoal-plaster was placed in the bottom.

In the tests for fecundity, 25 adult *S. caeca* were transferred to each of 12 such culture jars. These had been kept for 1 week without food in a larger culture jar that had been freshly prepared. Three diets, bakers' dried yeast, nematodes, and no food were tested. The no-food tests were included to determine what effect the fresh culture conditions might have on the egg-laying response. Periodically the eggs were counted and the food replaced, until young Collembola began to emerge.

Fifty first-instar young of *S. caeca* were transferred to each of 24 culture jars for investigation of the growth rate. These Collembola were less than 48 hours old and had not been fed, although some had ingested the charcoal-plaster substrate of the hatching jars. The appropriate test food was placed on a charcoal-plaster disk. Old disks were replaced every 2 days with freshly prepared disks in order to reduce fungal contamination. Every sixth day, until after eggs were produced and second generation young hatched, the length from anus to the anterior margin of the mesonotum was measured.

Measurement was accomplished by transferring specimens to a 1-dram shell vial. The vial contained a layer of charcoal-plaster, 25 mm deep, which provided a dark background against which to measure the light-colored Collembola, and which raised the bottom to within the working distance of the microscope that was used. The Collembola were anesthetized with CO_2 as described. The vial was covered with a glass cover slip which prevented escape of the CO_2 and kept the specimens anesthetized long enough for measurements to be made with an ocular micrometer.

Two control diets, bakers' dried yeast and no food, were used in these tests. The no-food control was used to check the possibility, cited by Hale (1967), that the charcoal-plaster substrate could provide some nutrient, probably because of fungus growth on it.

The nematodes for the experimental diet were obtained in a manner that made them as free as possible from extraneous material. The suspension of nematodes that resulted from one extraction was used as the starting material for a second extraction. This resulted in a suspension of nematodes that was essentially free from potato particles or starch grains, but which still contained a small quantity of very fine debris. This suspension was then filtered using a nylon sieve with holes 35μ square; the nematodes that were retained on the sieve were again suspended in tap water. This final suspension was then filtered through the charcoal-plaster disks and the nematodes retained were fed to the Collembola. Since the fine particulate matter was much smaller than the smallest nematodes, which easily passed through the sieve, most of the debris also went through leaving fairly clean nematodes. Undoubtedly, some microorganisms remained associated with the nematodes; however, the volume of microorganisms was quite small in comparison with that of the nematodes.

Digestion of Nematodes

In only 5 of the 17 specimens dissected following direct observation was any recognizable evidence of nematodes found (table 6). This evidence usually consisted of the anterior end with the pharyngeal apparatus and the external cuticle. A few whole nematodes were found. However, results were inconsistent and the evidence gave no indication of the numbers of nematodes that had been eaten. Evidently most of the nematodes were broken or digested beyond recognition in the short time that it took to observe and to kill the Collembola.

When Collembola were given nematodes on the charcoal-plaster disks, those in the 13-hour group ate 384 per Collembola, while those in the 24-hour group ate 837 per

Table 6. Results of dissecting Collembola that were observed feeding on nematodes

Species	Time observed (minutes)	Number eaten	Nematodes in gut contents
E. dissimilis . . .	—	—†	none
E. dissimilis . . .	5	30	1 piece
E. dissimilis . . .	5	17	none
E. dissimilis . . .	5	8	none
E. dissimilis . . .	5	71	several pieces, a few whole nematodes
S. caeca	—	many‡	none
S. caeca	—	many‡	a few pieces
E. dissimilis . . .	15	18	a few pieces
S. caeca	15	12	none
S. caeca	10	13	a few pieces
S. caeca	—	—†	none
S. caeca	—	—†	none
S. caeca	—	—†	none
S. caeca	—	—†	a few questionable pieces
S. caeca	15	17	none
S. caeca	15	many‡	none
S. caeca	15	many‡	none

†Number eaten were not counted, but other specimens in the dish were observed eating nematodes.

‡Number eaten not counted, but this specimen was observed eating nematodes.

Collembola. In both cases the difference between the experimental and control averages was highly significant. However, no evidence of nematodes was found in the gut contents of any of these Collembola. Control dissections made from Collembola that had fed on yeast only showed that the gut contents appeared to be the same as the gut contents of those that had fed on nematodes. In contrast, a group of nematodes that had been killed in hot water and squashed in glycerine on a slide were still easily recognizable as nematodes, even though they were broken and distorted.

Fecundity and Growth Rate

Data from the tests on fecundity are presented for the number of eggs present on the 8th day, 2 days before the young emerged, and the number present on the 11th day (table 7). The 2 foods produced similar results and, although some eggs were produced in jars with no food, the new culture conditions and stored food reserves were responsible for only a small percentage of the eggs.

In all cultures of the studies on growth rate, except for 1, and excluding those with a no food diet, more than 50 eggs were present on the 18th day; on the 24th day young began to emerge. By the 26th day all cultures except the one had produced young and had many more eggs which appeared ready to hatch. The exception to this was one of the jars with a nematode diet. Eggs were produced, but they never developed to the stage during which the outer chorion ruptured, an event that occurred in the other cultures between the second and third day after the eggs were layed. This same response, failure of eggs to develop, was seen in cultures in other portions of this work and possibly is related to contamination. Since the growth of the Collembola did not seem to be different from other similar cultures, the measurements were included in the statistical treatment.

Table 7. Eggs produced by adult *S. caeca* fed 3 diets

	Number of eggs (±Sd) †			LSD‡ $\alpha = .05$
	Yeast	Nematodes	No food	
Day 8 . .	196 (±48.9)	183 (±41.1)	52 (±24.1)	51.1
Day 11 . .	296 (±83.6)	292 (±69.7)	37 (±11.3)	81.9

†Four replicates each treatment, 25 adults per treatment.

‡Difference between averages greater than the LSD value indicates significance.

Specimens from the no food replicates grew very little and more than 30 per jar died during the experiment. It is thus concluded that the substrate provided very little nutrient, if any. Those reared on nematodes grew more rapidly and attained a larger average size than those reared on yeast (figure 1). *S. caeca* fed only nematodes were 0.087 mm longer at the time of the final measurement than those fed yeast. The calculated t value for a comparison of these average lengths is 3.90, which exceeds the tabulated $t_{(\alpha=0.01,\ df=144)}$ of 2.35, indicating a difference that is highly significant. Thus the diet of nematodes actually provided more rapid growth and a larger size at the time of emergence of the second generation.

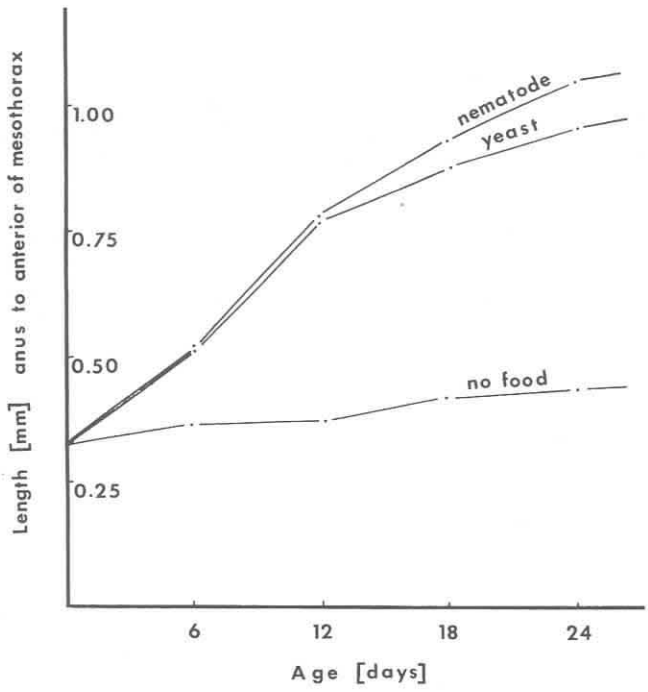

Figure 1. Growth of *S. caeca* fed 3 diets through 1 generation.

CONCLUSIONS AND DISCUSSION

In view of this evidence it is concluded that some Collembola are predatory on nematodes. Considering the variety of other evidence that Collembola are also herbivorous the conclusion must then be drawn that some Collembola, and possibly many Collembola, are omnivorous.

One objection to the hypothesis that Collembola feed on nematodes is that evidence of nematodes has not been found in examinations of gut contents (Gilmore, 1967;

Poole, 1959). However, the results of the dissections verifying the rapidity of the disappearance of nematode bodies from the gut contents of Collembola discounts such an objection. In the work of Poole (1957) specimens for gut content analysis were collected by Berlese funnel. All evidence of nematodes would have disappeared before the specimens finally fell into the fixative. Since most of my dissections failed to show evidence of nematodes even when made immediately after the specimen had been observed feeding on nematodes, natural populations of Collembola could not be expected to have identifiable nematode parts in their gut contents. This rapid disappearance of nematode parts makes it necessary to question the reliability of such gut content analyses. If a food element such as nematodes, which appears to be a satisfactory food source, is so readily digested, then one can question whether an item that remains visible in the gut contents provides any significant nutrition. The gut contents of Collembola usually include fungus spores and hyphae, items that are passed unchanged with the fecal pellets.

The controlled conditions of the charcoal-plaster disk method might also be cited as an objection to the hypothesis that Collembola eat nematodes. In a natural habitat the nematodes would have more chance of escaping from the Collembola. However, the method does answer the question for which it was designed, and answers it affirmatively. Several species of Collembola recognize nematodes as food and will consume large numbers of various species. The test using the soil-vermiculite mixture, while showing that the number consumed is reduced, also indicates that many nematodes would be eaten in a more natural environment. Considering the normal micro-environment of many soil nematodes (Wallace, 1963; Tribe, 1957) it seems likely that nematodes would be available to Collembola. This would be true of ectoparasitic nematodes, migrating immatures of endoparasites, and females or eggs of such endoparasites as *Heterodera* and *Meloidogyne*, as well as free living saprophytic forms.

The initial observations made before my research provide a particularly good example of the availability of nematodes in nature. The specimens involved (*E. dissimilis*) were observed feeding on immature nematodes which had migrated to high points in ant cultures. Nickle and Ayre (1966) and Markin and McCoy (1968) report the same behavior for immatures of nematodes that have been found in the pharyngeal glands of ants. These nematodes, *Caenorhabditis dolichura* (A. Schneider, 1866) Dougherty, and *Diploscapter lycostoma* Volk, are usually saprozoic, but the immatures are also found in these glands. Both reports on these nematodes point out that the immatures climbed to high points in cultures, on detritus, etc., and extended one end into the air. They then attached themselves to passing ants and either migrated or were transferred to the mouthparts by the cleaning operations of the ant. The *E. dissimilis* were observed eating immature nematodes that were fully exposed in the same manner.

Nickle and Ayre (1966) reported that up to 25 percent of field colonies of the ants, *Camponotus herculeanus* (L.) and *Acanthomyops claviger* (Roger), contained nematodes and that 100 percent of the individuals in cultures were infected after 2 months. Thus, it is evident that nematodes would be present in the field, and there is no reason to believe that their behavior would be any different than that exhibited in the cultures. Several Collembola have been collected from ant nests, including *E. dissimilis* (Christiansen, 1958). Both nematodes and Collembola would thus be in the same micro-environment with the nematodes exposed to predation. There is no reason to doubt that Collembola would eat the nematodes under these field conditions.

A major question raised by my work is the effect of predatory behavior of Collembola on a natural population of nematodes. Considering the number of nematodes that Collembola can eat and recorded estimates of population sizes of both groups, some answers can be suggested. Christiansen (1964) cites estimates of 1000 to 1,000,000 Collembola per cubic meter of soil, with an average of about 100,000. Hyman (1951) gives an estimate of 100 to 600 million nematodes per acre of soil in the United States, mostly in the top 6 inches, or up to 6 billion per acre of fertilized soil in China. Using even the highest number of nematodes and the average number of Collembola, one can obtain an estimate of 15 nematodes per Collembola under each square meter of soil surface. Both of these estimates are probably low. Nonetheless, a difference of 10 in the ratio between nematodes and Collembola would still result in an estimate of 150 nematodes per Collembola, which is within the order of magnitude of the number of nematodes eaten daily by Collembola in my experiments. Thus the presence of Collembola in soil could possibly make a substantial difference in a nematode population.

A limited amount of work has been done on other predators of nematodes. Most of this work has concentrated on predacious fungi (Wallace, 1963), but oribatid mites (Rockett and Woodring, 1966), tardigrades and protozoa (Doncaster and Hooper, 1961), staphilinid larvae and enchytraeids (Doncaster, 1962), and several groups of nematodes have been cited as predators on other nematodes. Rockett and Woodring (1966) found that the adults of *Pergalumna* sp. could consume 1 nematode in 75 seconds; the tritonymph required 40 seconds and the protonymph 20 minutes. In contrast, the maximum number eaten by 1 Collembola was 71 in 5 minutes or 1 every 4½ seconds.

This discussion is not meant to imply that Collembola alone could be effective in biological control of important nematode plant parasites, but it does seem that Collembola could have important effects on nematode popula-

tions when considered in conjunction with other factors. For example, the effect of various pesticides on the entire soil fauna should be considered. Some chemicals such as Sevin are highly toxic to Collembola (Aspock and Lan, 1963), although others are quite toxic to predators of Collembola, but do not affect Collembola (Edwards and Dennis, 1960). If a chemical deleterious to Collembola were used the effect could be to remove a portion of the natural control of nematodes, with the result of economically damaging populations. On the other hand, if a chemical that killed Collembola predators were used, the effect would be to permit a large increase in the number of Collembola, and a corresponding decrease in the number of nematodes. Such an increase in the size of the Collembola population actually occurred when DDT was used (Sheals, 1956; Menhinick, 1962).

REFERENCES

Aspock, H., and Lan, H. A.
1963. Ecological effects and physiological peculiarities of the plant protection chemical Sevin. Z. F. Angew. Zool. 50:343–80.

Brown, W. L., Jr.
1954. Collembola feeding on nematodes. Ecol. 35:421.

Christiansen, K.
1958. The nearctic members of the genus *Entomobrya* (Collembola). Mus. Comp. Zool. Bul. 118(7).
1964. Bionomics of Collembola. Ann. Rev. Entomol. 9:147–78.

Doncaster, C. C.
1962. Predators of soil nematodes. Parasitol. 52:19pp.

Doncaster, C. C., and Hooper, D. J.
1961. Nematodes attacked by protozoa and tardigrades. Nematol. 6:333–5.

Edwards, C. A.
1955. Simple techniques for rearing Collembola, Symphyla, and other small soil inhabiting arthropods. *In* Soil zoology, pp. 412–416, edited by D. K. McE. Kevan. Butterworths, London.

Edwards, C. A., and Dennis, E. B.
1960. Some effects of Aldrin and DDT on soil fauna of arable land. Nature 188:767.

Edwards, C. A., and Patton, R. L.
1965. Effects of carbon dioxide anesthesia on the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). Entomol. Soc. Amer. Ann. 58:828–32.

Englemann, M. D.
1966. Energetics, terrestrial field studies and animal productivity. *In* Advances in ecological research, pp 73–115, edited by J. B. Cragg. Academic Press, New York.
1968. The role of soil arthropods in community energetics. Amer. Zool. 8:61–9.

Gilmore, S. K.
1967. Foods ingested by *Tomocerus* spp. (Collembola: Entomobryidae) in relation to habitat. Thesis for degree of M. S., Cornell Univ., Ithaca, N. Y. Microfilmed.

Goto, H. E.
1960. Simple techniques for the rearing of Collembola and a note on the use of a fungistatic substance in the culture. Entomol. Mon. Mag. 96:138–40.

Hale, W. G.
1967. Collembola. *In* Soil biology, pp. 397–411, edited by A. Burges and F. Raw. Academic Press, New York.

Hyman, L. H.
1951. The invertebrates. Vol. 3. Acanthocephala, Aschelminthes, and Entoprocta: The Pseudocoelomate Bilateria. 572 pp. McGraw-Hill Co., New York.

Mai, W. F., Lyon, H. H., and Kruk, T. H.
1968. Pictorial key to genera of plant parasitic nematodes. Dept. Plant Path., N. Y. State Coll. Agr., Cornell Univ., Ithaca, N. Y. Mimeo. bul. and photo plates.

Markin, G. P., and McCoy, C. W.
1968. The occurrence of a nematode *Diploscapter lycostoma,* in the pharyngeal glands of the Argentine ant, Iridomyrmex humilis. Entomol. Soc. Amer. Ann. 61:505–9.

Menhinick, E. F.
1962. Comparison of invertebrate populations of soil and litter of mowed grasslands in areas treated and untreated with pesticides. Ecol. 43:556–61.

Murphy, P. W., and Doncaster, C. C.
1957. A culture method for soil meiofauna and its application to the study of nematode predators. Nematol. 2:202–14.

Nickle, W. R., and Ayre, G. L.
1966. *Caenorhabdidis dolichura* (A. Schneider, 1866) Dougherty (Rabditidae, Nematoda) in the head glands of the ants *Camponotus herculeanus* (L.) and *Acanthomyops claviger* (Roger) in Ontario. Entomol. Soc. Ont. Proc. 96:96–8.

Patton, R. L., Edwards, L. J., and Gilmore, S. K.
1968. Delivering safe levels of CO_2 for insect anesthesia. Entomol. Soc. Amer. Ann. 61:1046–7.

Poole, T. B.
1959. Studies on the food of Collembola in a Douglas fir plantation. Zool. Soc. Lond. Proc. 132: 71–82.

Rockett, C. L., and Woodring, J. P.
1966. Oribatid mites as predators of soil nematodes. Entomol. Soc. Amer. Ann. 59:669–71.

Sharma, G. D., and Kevan, D. K. McE.
1963. Observations on *Isotoma notabilis* (Collembola: Isotomidae) in eastern Canada. Pedobiol. 3: 34–47.

Sheals, J. G.
1956. Soil population studies I: The effect of cultivation and treatment with insecticides. Bul. Entomol. Res. 47:803–22.

Steele, R. G. D., and Torrie, J. H.
1960. Principles and procedures of statistics. 418 pp. McGraw Hill Co., New York.

Torne, E. von
1964. Über die anzucht und haltung individuenreicher Collembolenpopulationen. Pedobiol. 4:256–64.

Tribe, H. T.
1957. Ecology of microorganisms in soils observed during their development upon buried cellulose film. *In* Microbial ecology, pp. 287–98. Soc. Gen. Microbiol. Symp. 7. Cambridge Univ. Press.

Wallace, H. R.
1963. The biology of plant parasitic nematodes. 280 pp. Edward Arnold, London.

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